Eur J Vasc Endovasc Surg **27**, 608–616 (2004)
doi: 10.1016/j.ejvs.2004.01.020, available online at http://www.sciencedirect.com on SCIENCE DIRECT®

Cellular Engineering of Conduits for Coronary and Lower Limb Bypass Surgery: Role of Cell Attachment Peptides and Pre-conditioning in Optimising Smooth Muscle Cells (SMC) Adherence to Compliant Poly(carbonate–urea)urethane (MyoLink™) Scaffolds

S. T. Rashid,[1,2] H. J. Salacinski,[2] M. J. C. Button,[1] B. Fuller,[2] G. Hamilton[1,2] and A. M. Seifalian[1,2*]

[1]*Biomaterial and Tissue Engineering Centre (BTEC), Royal Free & University College Medical School, University College London, Royal Free Campus and* [2]*Vascular Unit, University Department of Surgery, Royal Free Hospital, Hampstead NHS Trust, London, UK*

Objective. *We are developing a hybrid arterial bypass graft of compliant poly(carbonate–urea)urethane (MyoLink™), endothelial and smooth muscle cells (SMCs). To enhance adhesion of SMCs we assessed various attachment factors and the effect of pre-conditioning on cell retention.*
Methods. *MyoLink segments were coated with either RGD, superfibronectin, fibronectin, fibronectin-like engineered polymer protein (FEPP), FEPP plus or type 1 collagen overnight.* ^{111}*Indium-radiolabelled SMCs were placed onto MyoLink segments for 48 h before being aspirated, then lavaged off. All grafts, aspirates and lavages were counted in a gamma counter. SMC viability on the MyoLink segments was also assessed for viability using the Alamar blue redox assay. Separately, MyoLink grafts lined with radiolabelled SMCs were divided into a pre-conditioned group, exposed to subarterial pulsatile flow whilst another group were held in static culture. After 1-week, grafts were exposed to arterial pulsatile flow whilst radioactivity was assessed using a gamma camera.*
Results. *Only FEPP plus significantly enhanced SMC attachment: mean of 32 ± 6% cell attachment compared to 21 ± 5% for uncoated control. Cell viability was enhanced by all attachment factors except fibronectin. Pre-conditioning was shown to significantly enhance the retention of SMCs onto the MyoLink once exposed to pulsatile arterial flow: the final attachment was 57 ± 7% for the static and 76 ± 7% for the pre-conditioned group.*
Conclusions. *FEPP plus enhances SMC attachment to MyoLink. We believe this is because of its repeating sequences of RGD and its positive charge. Pre-conditioning enhances the retention of SMCs to MyoLink once exposed to pulsatile arterial flow.*

Key Words: Cell adhesion; Cell retention; Compliance; FEPP plus; Hybrid graft; Polyurethanes; RGD; Shear stress; Smooth muscle cell; Tissue engineering.

Introduction

Obstructive atherosclerotic vascular disease in the form of cardiovascular and cerebrovascular disease is the largest cause of mortality and morbidity in the USA and Europe.[1] When angioplasty or stenting of the occluded vessels is not possible or unsuccessful then the surgical treatment for coronary artery and lower limb ischaemia involve the use of bypass grafts. For the majority of interventions this involves the use of the long saphenous vein or even the cephalic and basilic veins harvested from the arm or leg of the patient. However, in about a third of patients this source proves inadequate or unsuitable.[2] Therefore surgeons have turned to prosthetic materials like polyethylene terephthalate (Dacron) and expanded polytetrafluoroethylene (PTFE). However, these prosthetic materials prove to be inferior to venous conduits, especially when the vessel diameter is under 5 mm, with lower patency and higher infection rates.[3–5]

There are two important causes of the high failure rates in current prosthetic grafts, namely mechanical failings such as compliance mismatch between native

*Corresponding author. Dr A. M. Seifalian, PhD, University Department of Surgery, Royal Free Hospital, Royal Free and University College Medical School, Pond Street, London NW3 2QG, UK
E-mail: A.Seifalian@RFC.UCL.AC.UK.

1078–5884/060608 + 09 $35.00/0 © 2004 Elsevier Ltd. All rights reserved.

artery and prosthetic graft[6–8] and biological failings including absence of antithrombogenic properties and a living, responsive tissue adaptive to changes in the local environment.[9]

In an attempt to improve this situation, research has focused on three strategies: (a) newer types of prosthetic material; (b) making the existing prosthetic materials more natural by lining the lumen of the graft with endothelial cells (seeding) and (c) growing completely new vascular conduits from autogenous material using tissue-engineering techniques.

In this study, we describe the development of a hybrid graft using compliant poly(carbonate-urea)urethane (MyoLink™) as a simple scaffold onto which SMCs and extra-cellular matrix (ECM) develop.

The MyoLink graft has a honeycomb structure that allows it to maintain compliance similar to that of human artery[10] and pulsatile flow *in vivo* through a mechanism of wall compression that accommodates increases in volume without the need for external dilation. This is an advance on previous polyurethane based grafts which exhibited a compliance mismatch caused by perivascular ingrowth.[11] MyoLink has been shown to have superior ability to attach endothelial cells (EC), which can be further improved by pre-lining the graft with attachment factors like collagen and fibronectin.[10–13] The graft has undergone *in vitro* degradation tests and has been implanted in a dog model for 36 months, demonstrating very high biostability.[14–16] This graft is already in use as an A–V fistula for haemodialysis access and is undergoing a phase I clinical trial as a peripheral vascular bypass graft.[14]

Essentially all peptides promoting cell adhesion are derived from sequences found in ECM molecules. Of these fibronectin (FN) and vitronectin are the primary serum glyoproteins promoting cell adhesion, and FN has been the most studied.[17] FN is a glycoprotein (~250,000 Da) with a modular structure. It contains domains for binding fibrin, heparin, gelatin, collagen, EC, and has cysteine and arginine-glycine-aspartate (RGD) residues. Critically this RGD amino acid sequence[18] in the 10th type III repeat of FN serves as a primary cell attachment cue.[19] RGD is the most studied and effective peptide for cell adhesion on synthetic surfaces, based upon its widespread distribution within organisms, its ability to address more than one cell adhesion receptor and its biological impact upon cell anchoring, behaviour and survival. Furthermore cell adhesive RGD sites have been found in other ECM proteins like vitronectin, collagen, fibrinogen, von Willebrand factor, laminin, membrane proteins and even viral and bacterial proteins.[20]

Integrins are the main cell receptors for ECM proteins like FN, laminin, collagens, vitronectin and so are the main mediators of cell–cell and cell–ECM adhesion.[19] Integrin–ECM interactions are highly promiscuous: there is evidence that upon binding different integrins, the same ECM protein (e.g. FN) can mediate different functions and one integrin can bind multiple ligands. Integrins are heterodimers of two noncovalently associated glycoproteins, α and β: there are 18α and 8β subunits combining to form at least 24 heterodimers.[21] About half have been shown to bind to ECM molecules in an RGD-dependent manner.[20]

Although FN significantly improves EC retention, fibronectin is susceptible to hydrolysis,[17] so we decided to compare FN to variations of FN and to compounds containing only the RGD peptide sequence as it was hypothesised that increasing the number of RGD motifs in engineered constructs would result in significant improvements in cell adhesion. Attachment factors tested were RGD itself; superfibronectin (SFN) where a fragment from the first type-III repeat of fibronectin is bound to fibronectin to produce disulphide cross-linking thus resulting in multimers of the RGD motif of high molecular mass;[22] fibronectin like engineered polymer protein (FEPP) a segmented copolymer consisting of amino acid sequence blocks modelled on the crystalline segments of silk fibroin and the RGD domain of human fibronectin[23] and fibronectin like engineered polymer protein plus (FEPP +) where multiple copies of the RGD sequence are engineered within a positively charged copolymer.[24]

Furthermore studies have shown that EC attachment can be enhanced by lining synthetic grafts with SMC first.[25,26]

Another factor shown to positively influence the development of tissue-engineered grafts is called 'pre-conditioning'. Here the vascular construct is exposed, *in vitro*, to pulsatile flow and pressure. This has been shown to enhance cell proliferation, tissue formation and mechanical properties.[27] Our laboratory has previously shown that pre-conditioning with physiological shear stress using a flow circuit[28–30] we have developed can significantly enhance EC retention, viability and morphology.

Our laboratory is on the process of developing a hybrid graft consisting of a compliant scaffold (MyoLink), SMCs and ECs sourced from the same patient. The first stage is to produce a SMC layer mimicking the arterial media, so that the eventual hybrid graft could be vasoreactive. This would have the added benefit of enhancing EC adherence compared to pure EC seeding of grafts (i.e. without SMCs). In order to have a viable SMC layer it must be able to resist the forces of arterial shear stress. Hence we investigated what impact pre-conditioning would have on

cell retention at arterial pressures and flows. Therefore, the aim of this study was to determine whether attachment factors, and specifically those with multiple repeats of the RGD sequence, could enhance the adhesion of SMCs to the MyoLink and to examine the effect on cell retention of our pre-conditioning technique.

Methods and Materials

Material chemistry and manufacture of poly(carbonate-urea)urethane 'MyoLink'

The chemistry and manufacture of this graft have been published.[31] Briefly, it is made of poly(carbonate-urea)urethane extruded *via* low temperature cast coagulation. All grafts were 4 mm in internal diameter.

Cell extraction and culture

Human umbilical vein and arterial smooth muscle cells (SMC) were extracted using a novel enzymatic extraction process developed in-house.[32] Briefly: segments of umbilical artery and vein were cleaned of adventitia and sequentially treated with various concentrations of collagenase and elastase. SMCs were serially cultured in SMC tissue culture medium. This medium consisted of DMEM (Dulbecco's modified Eagle's medium) with HEPES modification, 10% FBS (foetal bovine serum), 2 mM L-glutamine, 2.5 mcg/ml amphotericin B, 100 units/ml penicillin and 100 mcg/ml streptomycin. The SMCs were stained with α-actin to confirm their SMC status.

Cell radiolabelling

The cells were used when confluent on the sixth passage. After trypsinization and resuspension in SMC medium, a cell count was obtained and cells were radiolabelled according to a protocol we have developed[33] with 1.8 MBq ^{111}In-oxine (Amersham International, Amersham, Bucks, UK) per 10^6 cells (incubated at 37 °C, 15 min). The cells were subsequently diluted in SMC medium to the concentration necessary to achieve a seeding density of 2.25×10^5 cells/cm^2.

Lining MyoLink with attachment factors and radiolabelled SMCs

Lengths of MyoLink were filled with either: RGD (Arg-Gly-Asp) at 633 mcg/ml, superfibronectin at 42 mcg/ml, fibronectin at 118 mcg/ml, FEPP 118 mcg/ml, FEPP plus 133 mcg/ml or type 1 collagen at 1 mg/ml (all from Sigma-Aldrich). A control batch consisted of native graft without a coating. The grafts were then left for 24 h to allow the scaffolds to be soaked with the various attachment factors. At the end of the 24 h period, the excess of the attachment factors were poured off.

The next day the grafts were cut along their length and then into 1–2 cm long pieces. These MyoLink segments were then placed in 2 ml syringes with the plungers holding them in position and the MyoLink lumen facing upwards. The syringes were filled with 0.7 ml of SMC solution at 2.25×10^6 cells/ml.

After 48 h in an incubator, the solution was aspirated off and the MyoLink inner lumens were lavaged with sterile phosphate buffered saline (PBS). The aspirate and washings were collected in separate tubes. Then the radioactivity of all specimens was determined using a gamma counter.

From the counts, the percentage radioactivity of the MyoLink was calculated as below:

$$\%\text{Radioactivity on MyoLink} = \frac{\text{MyoLink} \times 100}{\text{MyoLink} + \text{Aspirate} + \text{Lavages} + \text{Syringe}}$$

The radioactivity reflected the number of SMCs.

Cell viability

Viability of seeded cells were assessed using Alamar blue™ (Serotec Ltd, Kidlington, Oxford, UK) assay, as per our previous protocol.[34]

Briefly, the MyoLink segments were inserted into wells of a 24-well plate and filled with 1 ml of 10% Alamar blue™ (Alamar blue diluted 1:10 in SMC medium) and spectroscopic analysis was performed at 4 h by removing a 50.0 μl aliquot into a 96-well plate and absorbance read spectroscopically at wavelengths of 570 and 630 nm (Labsystems Multiscan MS visible spectrophotometer). Segments were subjected to scanning electron microscopy (SEM) for the presence and morphological appearance of seeded cells.

Effect of shear force on cell retention by pulsatile arterial flow

We modified the validated flow system of Giudiceandrea *et al*.[33] to simulate *in vitro* the pulsatility and flow waveform including reverse flow, pressures, and degree of oxygenation and pH of physiological

femoral artery circulation *in vivo*. We used this model to accurately determine SMC adhesiveness and retention on grafts exposed to physiological shear stress.

To investigate the impact of pre-conditioning, eight grafts were lined with radiolabelled SMCs as per the above method. Four of the grafts were left in static culture and four were pre-conditioned in the above flow circuit.[12] In both experiments, the grafts were incubated at 37 °C and 5% CO_2. After a week the grafts were put into a pulsatile flow circuit and dynamic scintigraphy images were acquired using a gamma camera.[12,33,35]

Radioactivity from the ^{111}In-oxine-labelled SMCs was measured using a gamma camera scanner linked to an image processing system. The section of the circuit containing the vascular grafts was positioned over the gamma camera and imaged throughout the perfusion period. An on-line workstation recorded all images within 64 × 64 matrices. The initial six images were each acquired over 5 min, with 22 subsequent images acquired over a 15 min span and used to generate time-activity curves corrected for background, spontaneous indium leakage, and isotope decay (half-life of ^{111}In = 68 h). Cell attachment (CA) with respect to time was calculated from the equation below, where $(t)_n$ is time in minutes, $(t)_0$ is immediately before initiation of flow, and CPMG and CPMB are the counts per minute over the graft and background computed from analysis of dynamic scintigraphy images. Grafts were perfused for 8 h.

$$CA = \frac{\text{Cells}(t)_n}{\text{Cells}(t)_0} \times 100$$

$$= \frac{\text{CPMG}(t)_n - \text{CPMB}(t)_n}{\text{CPMG}(t)_n} \times 100$$

Data analysis and statistical methods

Experiments were repeated six times. Data are presented with mean ± standard deviation (SD). For the cell attachment and viability data, the different groups are compared using 1-way ANOVA test using Bonferroni's multiple comparison test. For the time-activity retention data, at each time point the two groups were compared using an unpaired *t*-test.

Results

Assessment of cell attachment

The impact of the various attachment factors on cell attachment are summarised in Fig. 1. FEPP + was significantly better than the native MyoLink ($p < 0.01$) in terms of attachment of the SMCs, with a mean of 32 ± 6% cell attachment compared to 21 ± 5% for the native graft as calculated by the radiolabelling method.

Cell viability

Alamar blue at 4 h showed that every attachment factor except FN resulted in significantly higher ($p < 0.01$) metabolic activity compared to native (Fig. 2).

Pre-conditioning

Before exposure of the cells to the shear force of pulsatile blood flow, there was 8% lower cell count as determined by radioactivity levels on the vascular grafts in the preconditioning group compared to the static group, but this was not statistically significant (303 ± 22 *vs*. 280 ± 35, $p = 0.106$).

Upon commencement of the pulsatile flow circuit, the percentage of original radioactivity was significantly lower in the static grafts (81 ± 3 *vs*. 93 ± 2 for pre-conditioned grafts) after only 5 min ($p < 0.0001$). When assessing absolute values, however, the difference only became significant after 60 min ($p = 0.02$) because of the higher initial values in the static group (Fig. 3). At the end of the study period of 8 h, the values had plateaued, with the final % values being a mean of 57 ± 7 for the static group and 76 ± 7 for the pre-conditioned group.

Discussion

Cell–cell and cell–ECM interactions through integrins and other cell surface adhesion receptors are important for SMC adhesion, migration, proliferation, contraction, differentiation and apoptosis.[36] Integrins are important in SMC adhesion to ECM proteins like collagen.[37] Vitronectin and fibronectin are ECM proteins that mediate attachment of SMCs to both natural scaffolds like collagen and synthetic scaffolds like polyesters of lactic and glycolic acid.[38] RGD is a tripeptide sequence (arginine-glycine-aspartate) found in extracellular matrix proteins like fibronectin. It is the binding motif for cell surface integrin receptors and has been investigated extensively.[39–43] Our laboratory has shown that RGD when covalently bonded to MyoLink and particularly when this was in association with heparin, significantly enhances the retention and viability of seeded ECs.[44]

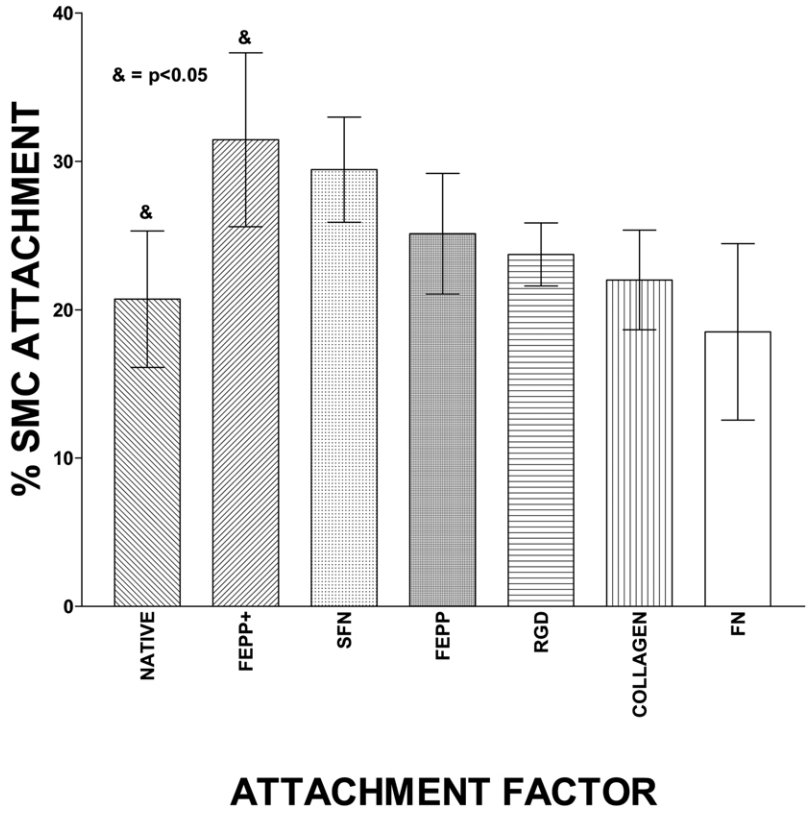

Fig. 1. SMC attachment with various attachment factors. Mean ± standard deviation. FN, fibronectin; SFN, superfibronectin; FEPP, fibronectin-like engineered polymer protein; FEPP + , fibronectin-like engineered polymer protein plus; RGD, Arg-Gly-Asp.

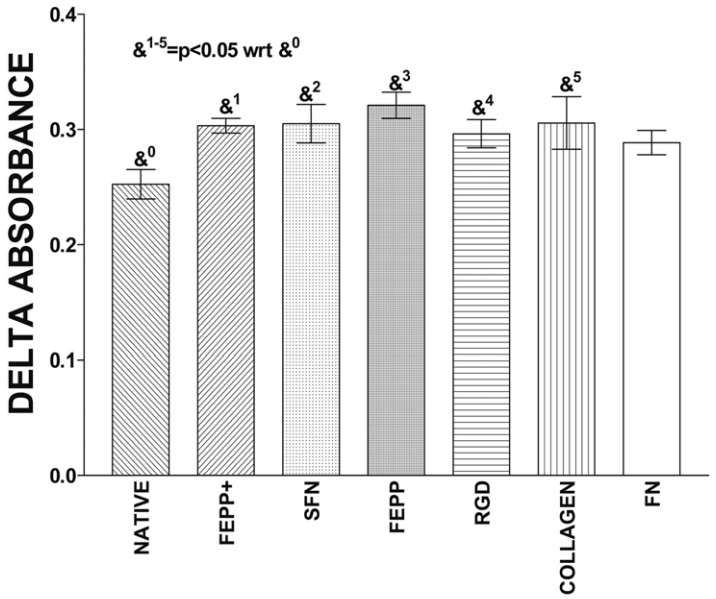

Fig. 2. Impact of various attachment factors on cell viability. Mean ± standard deviation. SFN, superfibronectin; FEPP, fibronectin-like engineered polymer protein; FEPP + , fibronectin-like engineered polymer protein plus; FN, fibronectin; RGD, Arg-Gly-Asp.

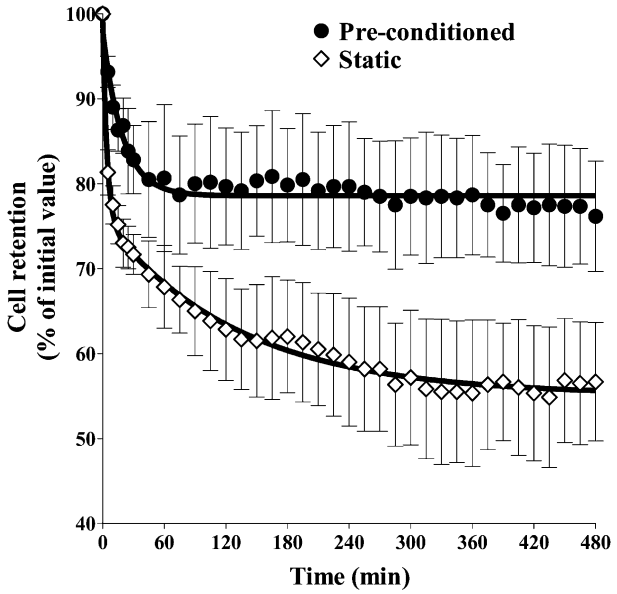

Fig. 3. Effect of pre-conditioning on SMC retention to MyoLink each value plotted is mean ± standard deviation.

We report a method for assessing attachment of SMCs to MyoLink using different attachment factors. By radiolabelling the SMCs, we have shown that the only type of attachment factor that significantly improves the attachment of SMCs compared to the native MyoLink graft is FEPP Plus.

Alamar blue at 4 h showed that there were more viable cells attached using any of the attachment factors, except FN, compared to native ($p < 0.01$).

Our belief is that the reason for the superiority of the FEPP plus is that it has a positive charge, which has been shown to attract the negatively-charged surface of cells[45,46] and it contains repeating sequences of RGD[47] which would enhance the binding of SMCs, principally through integrin receptors, to the MyoLink surface coated with RGD peptides. Further experiments are needed to determine if there is a time-dependent factor to the attachment.

Although coating the graft with attachment factors rather than bonding is a well-known methodology, it is true that this is not as reliable as bonding attachment factors to the graft. However, bonding is usually done for small peptides not larger repeats of peptide sequences. Furthermore, in our experience SMCs tend to grow into the pores of the honeycomb of poly(carbonate-urea)urethane. This compares to ECs, which sit on the surface. We have previously shown that bonding small peptide sequences with spacer arm technology improves EC attachment to the surface.[44] However, due to the amorphous crystalline nature of polyurethanes such bonding occurs post extrusion as an additional step and as such makes this procedure commercially less favourable as it is expensive and time consuming. If such a step is not required for SMC, which would not be confined to the graft surface, this makes the cost effectiveness of tissue-engineered hybrid constructs of EC and SMC commercially more acceptable. In addition, bonding is not a cost-effective technology for screening through a host of different attachment factors. Mechanical stress orientates SMCs and the ECM both in vitro or *in vivo*.[48–59] The exposure of tissue-engineered vessels to the mechanical stimulation of pulsatile flow results in increased mechanical strength of the constructs as measured by parameters like burst pressure.[29,30,60–63] This is critical if implanted vessels are to avoid aneurysmal dilation and ultimately rupture once exposed to long-term arterial blood pressures. The optimum conditions of the pre-conditioning are still to be defined but some propose that the ideal for tissue-engineered vessels may actually be fetal conditions[64] with low pulsatile pressure but high pulse rates. Furthermore exposure to shear stress has been shown to enhance endothelial cell attachment, retention and differentiation as well as being a critical factor in their biological regulatory function.[65–69] SMC proliferation also is enhanced by shear stress.[70] Although for this study shear stress was not measured, this variable is currently under investigation in our laboratory.

We have shown in this study that the use of pre-conditioning significantly enhances the retention of SMCs on MyoLink. This is not just a proliferative effect because rather than cell counts or metabolic assays we measured SMC numbers by radioactivity after having radiolabelled the SMCs at the start of the experiment. Therefore any proliferation could not increase the total amount of radioactivity and any decrease in radioactivity measured by the gamma camera could only be because of the detachment of radiolabelled SMCs. Indeed SEM confirms the superiority of pre-conditioned over static grafts (Fig. 4) with much greater cell coverage.

In conclusion, the goal of achieving a compliant hybrid graft has been partially realised by the development of the MyoLink scaffold which matches arterial compliance within the human blood pressure range.[13] This work has shown that pre-conditioning with pulsatile flow together with the use of FEPP plus as a peptide coating for the graft gives excellent SMC adhesion when exposed to shear stress. This work has only been taken to 8 h in the *in vitro* setting and further studies are focussed on long-term assessment *in vitro* and *in vivo*, of SMC attachment and the development of a neomedia.

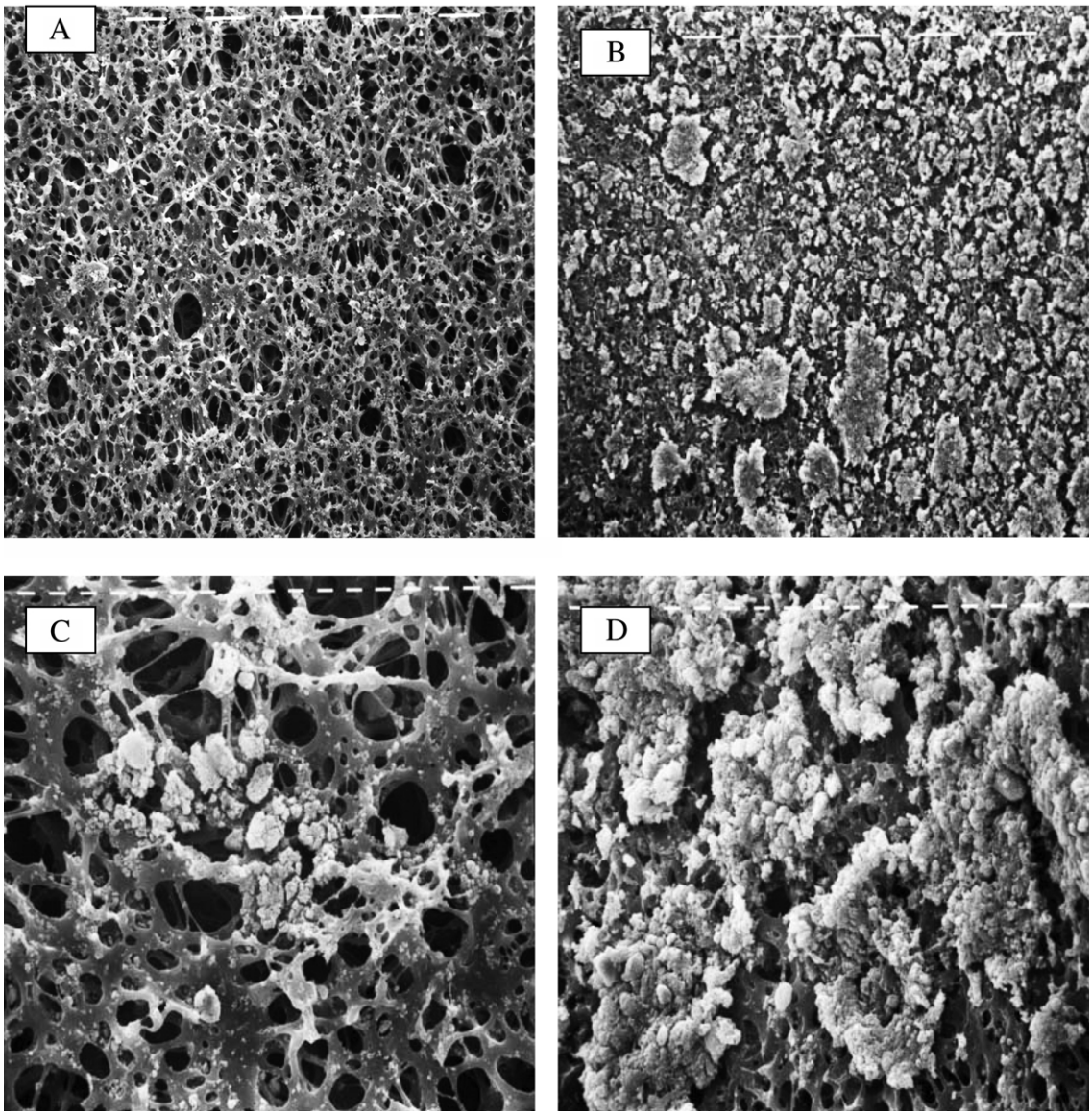

Fig. 4. (A–D) Scanning electron micrographs of SMCs on MyoLink grafts. LHS: Static MyoLink: (A) top: × 60.5; (C) bottom: × 242 magnification, RHS: Pre-conditioned MyoLink: (B) top: × 60.5; (D) bottom: × 242 magnification.

Acknowledgements

S.T.R. and H.J.S. received grants from Freemedic Ltd, UCL. Credent Ltd supplied MyoLink grafts.

References

1 World Health Organization. World Health Organization: The World Health Report 1999. 1-1-1999.
2 Veith FJ, Moss CM, Sprayregen S, Montefusco C. Preoperative saphenous venography in arterial reconstructive surgery of the lower extremity. *Surgery* 1979; **85**(3):253–256.
3 Bennion RS, Williams RA, Stabile BE, Fox MA, Owens ML, Wilson SE. Patency of autogenous saphenous vein versus polytetrafluoroethylene grafts in femoropopliteal bypass for advanced ischemia of the extremity. *Surg Gynecol Obstet* 1985; **160**(3):239–242.
4 Pourdeyhimi B, Wagner D. On the correlation between the failure of vascular grafts and their structural and material properties: a critical analysis. *J Biomed Mater Res* 1986; **20**(3): 375–409.
5 Abbott WM, Callow A, Moore W, Rutherford R, Veith F, Weinberg S. Evaluation and performance standards for arterial prostheses. *J Vasc Surg* 1993; **17**(4):746–756.
6 Abbott WM, Megerman J, Hasson JE, L'Italien G, Warnock DF. Effect of compliance mismatch on vascular graft patency. *J Vasc Surg* 1987; **5**:376–382.
7 Greenwald SE, Berry CL. Improving vascular grafts: the importance of mechanical and haemodynamic properties. *J Pathol* 2000; **190**(3):292–299.
8 Salacinski HJ, Goldner S, Giudiceandrea A, Hamilton G, Seifalian AM, Edwards A *et al*. The mechanical behavior of vascular grafts: a review. *J Biomater Appl* 2001; **15**(3):241–278.
9 Parikh SA, Edelman ER. Endothelial cell delivery for

cardiovascular therapy (Review) (217 refs). *Adv Drug Deliv Rev* 2000; **42**(1–2):139–161.
10 Stansby G, Berwanger C, Shukla N, Schmitz-Rixen T, Hamilton G. Endothelial seeding of compliant polyurethane vascular graft material. *Br J Surg* 1994; **81**(9):1286–1289.
11 Salacinski HJ, Punshon G, Krijgsman B, Hamilton G, Seifalian AM. A hybrid compliant vascular graft seeded with microvascular endothelial cells extracted from human omentum. *Artif Organs* 2001; **25**(12):974–982.
12 Giudiceandrea A, Seifalian AM, Krijgsman B, Hamilton G. Effect of prolonged pulsatile shear stress *in vitro* on endothelial cell seeded PTFE and compliant polyurethane vascular grafts. *Eur J Vasc Endovasc Surg* 1998; **15**(2):147–154.
13 Tai NR, Salacinski HJ, Edwards A, Hamilton G, Seifalian AM. Compliance properties of conduits used in vascular reconstruction. *Br J Surg* 2000; **87**(11):1516–1524.
14 Seifalian AM, Salacinski HJ, Tiwari A, Edwards A, Bowald S, Hamilton G. In vivo biostability of a poly(carbonate-urea)urethane graft. *Biomaterials* 2003; **24**(14):2549–2557.
15 Tiwari A, Salacinski H, Seifalian AM, Hamilton G. New prostheses for use in bypass grafts with special emphasis on polyurethanes. *Cardiovasc Surg* 2002; **10**(3):191–197.
16 Salacinski HJ, Tai NR, Carson RJ, Edwards A, Hamilton G, Seifalian AM. *In vitro* stability of a novel compliant poly(carbonate-urea)urethane to oxidative and hydrolytic stress. *J Biomed Mater Res* 2002; **59**(2):207–218.
17 Hynes RO. Fibronectins. New York: Springer, 1990.
18 Pierschbacher MD, Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* 1984; **309**(5963):30–33.
19 LeBaron RG, Athanasiou KA. Extracellular matrix cell adhesion peptides: functional applications in orthopedic materials. *Tissue Eng* 2000; **6**(2):85–103.
20 Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 2003; **24**(24):4385–4415.
21 Ruegg C, Mariotti A. Vascular integrins: pleiotropic adhesion and signaling molecules in vascular homeostasis and angiogenesis. *Cell Mol Life Sci* 2003; **60**(6):1135–1157.
22 Morla A, Zhang Z, Ruoslahti E. Superfibronectin is a functionally distinct form of fibronectin. *Nature* 1994; **367**(6459): 193–196.
23 Anderson JP, Cappello J, Martin DC. Morphology and primary crystal structure of a silk-like protein polymer synthesized by genetically engineered *Escherichia coli* bacteria. *Biopolymers* 1994; **34**(8):1049–1058.
24 Yang Y, Cardarelli PM, Lehnert K, Rowland S, Krissansen GW. LPAM-1 (integrin alpha 4 beta 7)-ligand binding: overlapping binding sites recognizing VCAM-1, MAdCAM-1 and CS-1 are blocked by fibrinogen, a fibronectin-like polymer and RGD-like cyclic peptides. *Eur J Immunol* 1998; **28**(3):995–1004.
25 Yu H, Wang Y, Eton D, Rowe VL, Terramani TT, Cramer DV *et al.* Dual cell seeding and the use of zymogen tissue plasminogen activator to improve cell retention on polytetrafluoroethylene grafts. *J Vasc Surg* 2001; **34**(2):337–343.
26 Yu H, Dai W, Yang Z, Kirkman P, Weaver FA, Eton D *et al.* Smooth muscle cells improve endothelial cell retention on polytetrafluoroethylene grafts in vivo. *J Vasc Surg* 2003; **38**(3): 557–563.
27 Hoerstrup SP, Zund G, Sodian R, Schnell AM, Grunenfelder J, Turina MI. Tissue engineering of small caliber vascular grafts. *Eur J Cardiothorac Surg* 2001; **20**(1):164–169.
28 Niklason LE. Techview: medical technology. Replacement arteries made to order. *Science* 1999; **286**(5444):1493–1494.
29 Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R *et al.* Functional arteries grown in vitro [see comments]. *Science* 1999; **284**(5413):489–493.
30 Niklason LE, Abbott W, Gao J, Klagges B, Hirschi KK, Ulubayram K *et al.* Morphologic and mechanical characteristics of engineered bovine arteries. *J Vasc Surg* 2001; **33**(3):628–638.
31 Edwards A, Carson RJ, Bowald S, Quist WC. Development of a microporous compliant small bore vascular graft. *J Biomater Appl* 1995; **10**(2):171–187.
32 Button M, Barden G, Fuller B, Hamilton G. Optimized extraction of smooth muscle cells from human vein for use in a tissue engineered vascular graft. *Cardiovasc Pathol* 2002; **11**(1):39. Abstract.
33 Giudiceandra A, Salacinski H, Tai N, Punshon G, Hamilton G, Seifalian A. Development and evaluation of an ideal flow circuit: assessing the dynamic behavior of endothelial cell seeded grafts. *J Artif Organs* 2000; **3**:16–24.
34 Seifalian AM, Salacinski HJ, Punshon G, Krijgsman B, Hamilton G. A new technique for measuring the cell growth and metabolism of endothelial cells seeded on vascular prostheses. *J Biomed Mater Res* 2001; **55**(4):637–644.
35 Salacinski HJ, Tai NR, Punshon G, Giudiceandrea A, Hamilton G, Seifalian AM. Optimal endothelialisation of a new compliant poly(carbonate-urea)urethane vascular graft with effect of physiological shear stress [in process citation]. *Eur J Vasc Endovasc Surg* 2000; **20**(4):342–352.
36 Moiseeva EP. Adhesion receptors of vascular smooth muscle cells and their functions. *Cardiovasc Res* 2001; **52**(3):372–386.
37 Ogle BM, Mooradian DL. The role of vascular smooth muscle cell integrins in the compaction and mechanical strengthening of a tissue-engineered blood vessel. *Tissue Eng* 1999; **5**(4):387–402.
38 Nikolovski J, Mooney DJ. Smooth muscle cell adhesion to tissue engineering scaffolds. *Biomaterials* 2000; **21**(20):2025–2032.
39 Mann BK, Tsai AT, Scott-Burden T, West JL. Modification of surfaces with cell adhesion peptides alters extracellular matrix deposition. *Biomaterials* 1999; **20**(23–24):2281–2286.
40 Walluscheck KP, Steinhoff G, Kelm S, Haverich A. Improved endothelial cell attachment on ePTFE vascular grafts pretreated with synthetic RGD-containing peptides. *Eur J Vasc Endovasc Surg* 1996; **12**(3):321–330.
41 Walluscheck KP, Steinhoff G, Haverich A. Endothelial cell seeding of de-endothelialised human arteries: improvement by adhesion molecule induction and flow-seeding technology. *Eur J Vasc Endovasc Surg* 1996; **12**(1):46–53.
42 Salacinski HJ, Tiwari A, Hamilton G, Seifalian AM. Cellular engineering of vascular bypass grafts: role of chemical coatings for enhancing endothelial cell attachment. *Med Biol Eng Comput* 2001; **39**(6):609–618.
43 Mann BK, West JL. Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds. *J Biomed Mater Res* 2002; **60**(1):86–93.
44 Tiwari A, Salacinski HJ, Punshon G, Hamilton G, Seifalian AM. Development of a hybrid cardiovascular graft using a tissue engineering approach. *FASEB J* 2002; **16**(8):791–796.
45 Ramamurthi A, Vesely I. Smooth muscle cell adhesion on crosslinked hyaluronan gels. *J Biomed Mater Res* 2002; **60**(1): 195–205.
46 Koyano T, Minoura N, Nagura M, Kobayashi K. Attachment and growth of cultured fibroblast cells on PVA/chitosan—blended hydrogels. *J Biomed Mater Res* 1998; **39**(3):486–490.
47 Krijgsman B, Seifalian AM, Salacinski HJ, Tai NR, Punshon G, Fuller BJ *et al.* An assessment of covalent grafting of RGD peptides to the surface of a compliant poly(carbonate-urea)urethane vascular conduit versus conventional biological coatings: its role in enhancing cellular retention. *Tissue Eng* 2002; **8**(4): 673–680.
48 Dartsch PC, Hammerle H, Betz E. Orientation of cultured arterial smooth muscle cells growing on cyclically stretched substrates. *Acta Anatomica* 1986; **125**(2):108–113.
49 Buck RC. Behaviour of vascular smooth muscle cells during repeated stretching of the substratum in vitro. *Atherosclerosis* 1983; **46**:217–233.
50 Kanda K, Matsuda T, Oka T. Two-dimensional orientational response of smooth muscle cells to cyclic stretching. *ASAIO J* 1992; **38**(3):M382–M385.
51 Kobashi T, Matsuda T. Fabrication of branched hybrid vascular prostheses. *Tissue Eng* 1999; **5**(6):515–524.

52 Ishibashi K, Kawazoe K, Matsuda T. Development of a hybrid vascular graft hierarchically layered three cell types. *Jpn J Artif Organs* 1996; **25**(3):733–737.
53 Hirai J, Matsuda T. Venous reconstruction using hybrid vascular tissue composed of vascular cells and collagen: tissue regeneration process. *Cell Transplant* 1996; **5**(1):93–105.
54 Matsuda T, Miwa H. A hybrid vascular model biomimicking the hierarchic structure of arterial wall: neointimal stability and neoarterial regeneration process under arterial circulation. *J Thoracic Cardiovasc Surg* 1995; **110**(4I):988–997.
55 Ishibashi K, Matsuda T. Reconstruction of a hybrid vascular graft hierarchically layered with three cell types. *ASAIO J* 1994; **40**(3):M284–M290.
56 Miwa H, Matsuda T, Iida F. Development of a hierarchically structured hybrid vascular graft biomimicking natural arteries. *ASAIO J* 1993; **39**(3):M273–M277.
57 Kanda K, Matsuda T. In vitro reconstruction of hybrid arterial media with molecular and cellular orientations. *Cell Transplant* 1994; **3**(6):537–545.
58 Kanda K, Matsuda T. Mechanical stress-induced orientation and ultrastructural change of smooth muscle cells cultured in three-dimensional collagen lattices. *Cell Transplant* 1994; **3**(6): 481–492.
59 Kanda K, Matsuda T. Behavior of arterial wall cells cultured on periodically stretched substrates. *Cell Transplant* 1993; **2**(6): 475–484.
60 Ziegler T, Nerem RM. Tissue engineering a blood vessel: regulation of vascular biology by mechanical stresses (see comments) (Review) (49 refs). *J Cell Biochem* 1994; **56**(2):204–209.
61 Nerem RM. Tissue engineering a blood vessel substitute: the role of biomechanics. *Yonsei Med J* 2000; **41**(6):735–739.
62 Seliktar D, Black RA, Vito RP, Nerem RM. Dynamic mechanical conditioning of collagen-gel blood vessel constructs induces remodeling in vitro. *Ann Biomed Eng* 2000; **28**(4): 351–362.
63 L'Heureux N, Pâquet S, Labbé R, Germain L, Auger FA. A completely biological tissue-engineered human blood vessel [see comments]. *FASEB J* 1998; **12**(1):47–56.
64 Stock UA, Vacanti JP. Cardiovascular physiology during fetal development and implications for tissue engineering. *Tissue Eng* 2001; **7**(1):1–7.
65 Dardik A, Liu A, Ballermann BJ. Chronic in vitro shear stress stimulates endothelial cell retention on prosthetic vascular grafts and reduces subsequent in vivo neointimal thickness. *J Vasc Surg* 1999; **29**(1):157–167.
66 Ott MJ, Ballermann BJ. Shear stress-conditioned, endothelial cell-seeded vascular grafts: improved cell adherence in response to in vitro shear stress. *Surgery* 1995; **117**(3):334–339.
67 Ballermann BJ, Ott MJ. Adhesion and differentiation of endothelial cells by exposure to chronic shear stress: a vascular graft model[Review] [33 refs]. *Blood Purif* 1995; **13**(3–4):125–134.
68 Fisher AB, Chien S, Barakat AI, Nerem RM. Endothelial cellular response to altered shear stress. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**(3):L529–L533.
69 Wu MH, Kouchi Y, Onuki Y, Shi Q, Yoshida H, Kaplan S *et al.* Effect of differential shear stress on platelet aggregation, surface thrombosis, and endothelialization of bilateral carotid-femoral grafts in the dog. *J Vasc Surg* 1995; **22**(4):382–390.
70 Shigematsu K, Yasuhara H, Shigematsu H, Muto T. Direct and indirect effects of pulsatile shear stress on the smooth muscle cell. *Inter Angio* 2000; **19**(1):39–46.

Accepted 13 January 2004